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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Avner YAYON et al.	Confirmation No.: 3532
Patent No.: 7,009,039 B2	Application No.: 10/761,615
Patent Date: March 7, 2006	Filing Date: January 20, 2004
For: PLASMA PROTEIN MATRICES AND METHODS FOR THEIR PREPARATION	Attorney Docket No.: 81408-4600

REQUEST FOR CERTIFICATE OF CORRECTION
UNDER 37 C.F.R. §§ 1.322 and 1.323

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Certificate
MAR 28 2006
of Correction

Sir:

Patentees hereby respectfully request the issuance of a Certificate of Correction in connection with the above-identified patent. The corrections are listed on the attached Form PTO-1050. The corrections requested are as follows:

Title Page:

Item (56), References Cited, Other Publications:

"Sims, C. Derek M.D. et al." reference, delete "Boston and Cambridge, Mass." and insert -- Plastic & Reconstructive Surgery --.

"A. Haisch et al." reference, after "Medical & Biological" delete "Engineerin" and insert -- Engineering --.

Column 24:

Line 49 (claim 1, line 4), delete "lplasma" and insert -- plasma --.

Line 51 (claim 1, line 6), delete "substontial" and insert -- substantial --.

Support for these changes appear in application claim 1, as amended on September 19, 2005.

03/27/2006 MBEYENE1 00000027 501814 7009039
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DC:460833.1

MAR 29 2006

Column 25:

Line 8 (claim 8, line 1), after "claim 7" delete "wherein the". This change is requested merely to correct an inadvertent clerical error.

Line 62 (claim 23, line 2), before "with thrombin in the presence of" insert -- are mixed --. Support for this change appears in application claim 23, as amended on September 19, 2005.

Column 26:

Line 2, delete "shape;" and insert -- shape --. Support for this change appears in application claim 23, as amended on September 19, 2005.

The requested changes are to correct errors of a clerical or typographical nature and do not involve changes that would constitute new matter or require reexamination.

A fee of $100 is believed to be due for this request. Please charge the required fees to Winston & Strawn LLP Deposit Account No. 50-1814. Please issue a Certificate of Correction in due course.

Respectfully submitted,

3-23-06
Date

Allan A. Fanucci, Reg. No. 30,256

WINSTON & STRAWN LLP
Customer No. 28765

212-294-3311

MAR 29 2006

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO.: 7,009,039 B2
DATED: March 7, 2006
INVENTORS: Yayon et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Title Page:
Item (56), References Cited, Other Publications:
"Sims, C. Derek M.D. et al." reference, delete "Boston and Cambridge, Mass." and insert -- Plastic & Reconstructive Surgery --.
"A. Haisch et al." reference, after "Medical & Biological" delete "Engineerin" and insert -- Engineering --.

Column 24:
Line 49, delete "lplasma" and insert -- plasma --.
Line 51, delete "substontial" and insert -- substantial --.

Column 25:
Line 8, after "claim 7" delete "wherein the".
Line 62, before "with thrombin in the presence of" insert -- are mixed --.

Column 26:
Line 2, delete "shape;" and insert -- shape --.

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(12) **United States Patent**
Yayon et al.

(10) **Patent No.: US 7,009,039 B2**
(45) **Date of Patent: Mar. 7, 2006**

(54) **PLASMA PROTEIN MATRICES AND METHODS FOR THEIR PREPARATION**

(75) Inventors: **Avner Yayon**, Moshav Sitria (IL); **Rachel Glicklis**, Lehavim (IL)

(73) Assignee: **ProChon Biotech Ltd.**, Rehovot (IL)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 40 days.

(21) Appl. No.: **10/761,615**

(22) Filed: **Jan. 20, 2004**

(65) **Prior Publication Data**

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Related U.S. Application Data

(63) Continuation of application No. PCT/IL02/00589, filed on Jul. 18, 2002.

(30) **Foreign Application Priority Data**

Jul. 19, 2001 (IL) 144446

(51) **Int. Cl.**
A61K 35/14 (2006.01)
A61K 38/00 (2006.01)

(52) **U.S. Cl.** **530/381**; 424/93.7; 424/422; 602/48; 514/12

(58) **Field of Classification Search** 424/93.7, 424/400, 422; 602/48; 514/12; 530/381
See application file for complete search history.

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(74) *Attorney, Agent, or Firm*—Winston & Strawn LLP

(57) **ABSTRACT**

A freeze dried biocompatible matrix comprising plasma proteins, useful as implants for tissue engineering as well as in biotechnology, and methods of producing the matrix are provided. Mechanical and physical parameters can be controlled by use of auxiliary components or additives which may be removed after the matrix is formed in order to improve the biological properties of the matrix. The matrices according to the present invention may be used clinically per se, or as a cell-bearing implant.

53 Claims, 7 Drawing Sheets

TABLE 3-continued

Goat	Treatment	Time	Results
			proliferation in and around wound.
945	Fresh sponges	10 weeks	No inflammation; mesenchymal cell proliferation
183	Collagen sponges	10 weeks	Massive inflammation
930	60° C. sponges	10 weeks	Minimal inflammation; Mesenchymal cell proliferation.

FIG. 7 shows the knee joints of the goats following the experiment.

Example 11

One-Step Procedure for Treating Damaged Cartilage: Suitable for Arthroscopy or Hemi-Arthrotomy

Autologous chondrocyte implantation has proven clinically effective in restoring hyaline-like cartilage to isolated chondral defects of the knee. The present therapies include three major steps:

1. Diagnostic Arthroscopy and biopsy of healthy cartilage.

2. Cultivation of cells.

3. Injection of cultured chondrocytes into the lesion under a periosteal flap, which is taken from the tibia and sutured over the lesion.

A variation of this technique provides incorporation of cells into a biodegradable material, including the matrix of the present invention. A less traumatic method is presented herein, wherein the patient undergoes a single surgical procedure for cartilage repair.

Procedure

A patient with a cartilage defect is called to the physician's office for a consultation several days prior to the arthroscopy or hemi-arthrotomy. Blood (approximately 100–250 ml) is drawn and plasma proteins are isolated. A plasma protein matrix, or several matrices, is prepared, labeled and stored aseptically until the day of the surgery.

On the day of the surgery, a small piece of healthy cartilage the patient's joint is removed, cut into small pieces and placed in a test tube containing collagenase, hyaluronidase or other cartilage degrading enzymes, or combinations thereof.

In the meantime, the surgeon will treat the defective region of the joint by removing damaged tissue, cleansing and preparing the area for an implant The prepared matrix is removed from its container and cut to fit the defective domain. Following approximately 20–30 minutes of enzymatic treatment, the cells and small pieces of cartilage are spun down in a tabletop centrifuge, rinsed in PBS and resuspended in a small amount (50 ul–1000 ul) of PBS. The surgeon seeds the cells onto the sponge, in situ. Alternatively the cells are absorbed into the sponge and the cell-bearing sponge implanted into the defective joint region. Optionally, extracellular matrix degrading enzymes and or other bioactive agents including growth factors and/or anti-inflammatory compounds are added to the sponge. In certain instances the surgeon will place a dry sponge directly onto the injured area, optionally add enzyme solution to said sponge and place a second, cell-bearing sponge on top of the first sponge. The joint is closed and is treated as customary for an arthroscopic or hemi-arthrotomy procedure and the patient is released to recover.

Kit

A kit comprising the components useful for practicing the method of the invention, will allow for the convenient practice of the method of the invention in a surgical setting. In a preferred embodiment, a kit of the invention will provide sterile components suitable for easy use in the surgical environment including, sterile solutions (saline, enzymes) a sterile, cell-free matrix material suitable for supporting autologous chondrocytes that are to be implanted into an articular joint surface defect and instructions for use. Although the matrix may be of any material that is biocompatible, non-immunogenic and has the ability to maintain cell growth and proliferation, the matrix is preferably prepared from allogeneic plasma, more preferably from autologous plasma

Example 12

Release of Bioactive Agents

One factor which may facilitate the development of tissues on the matrices is the delivery of growth factors or other biological agents into the local environment The incorporation and release of growth factors from these matrices is assessed in vitro or in vivo using radiolabeled or tagged growth factors, for example fluorescent-labeled, alkaline phosphatase labeled or horseradish peroxidase-labeled growth factor. The fraction and rate of released agent is measured by following the radioactivity, fluorescence, enzymatic activity or other attributes of the tag. Similarly, release of enzymes from the matrix is determined by analyzing enzymatic activity into the microenvironment in an in vitro or in vivo assay.

While the present invention has been particularly described, persons skilled in the art will appreciate that many variations and modifications can be made. Therefore, the invention is not to be construed as restricted to the particularly described embodiments, rather the scope, spirit and concept of the invention will be more readily understood by reference to the claims which follow.

What is claimed is:

1. An elastic freeze-dried biocompatible porous fibrin matrix useful as a scaffold for growing cells, wherein the matrix has substantially regular pores and a residual moisture below 3% and is obtained by mixing plasma proteins comprising fibrinogen and Factor XIII with thrombin and at least one anti-fibrinolytic agent in substontial absence of organic chelating agents.

plasma

substantial

2. The matrix according to claim 1 wherein the plasma proteins are present with at least 0.5 units of thrombin per milligram of protein.

3. The matrix according to claim 1 wherein at least one of the plasma proteins is autologous to a patient in need of the matrix.

4. The matrix according to claim 1 wherein all the plasma proteins are autologous to a patient in need of the matrix.

5. The matrix according to claim 1 wherein the anti-fibrinolytic agent is tranexamic acid.

6. The matrix according to claim 1 further comprising at least one auxiliary component selected from the group consisting of polysaccharides, anionic polysaccharides, glycosaminoglycans, or synthetic polymers.

7. The matrix according to claim 6 wherein the auxiliary component is selected from a group consisting of hyaluronic acid, pectin, alginate, galactans, galactomannans, glucomannans, polyuronic acids, heparin, chondroitin sulfate, dextran sulfate, dermatan sulfate, heparan sulfate, keratan sulfate, hexuronyl hexosaminoglycan sulfate, inositol hexasulfate, sucrose octasulfate and polyethylene glycol.

8. The matrix according to claim 7 ~~wherein the~~ wherein the auxiliary component is dextran sulfate or hyaluronic acid.

9. The matrix according to claim 1 wherein the cells are stem cells or progenitor cells.

10. The matrix according to claim 1 wherein the cells are selected from the group consisting of chondrocytes, osteocytes, hepatocytes and mesenchymal, epithelial, urothelial, neuronal, pancreatic, renal and ocular cell types.

11. The matrix according to claim 1 wherein the cells attain a density of at least 10^4 cells per cm^3.

12. The matrix according to claim 1 further comprising at least one bioactive agent, selected from the group consisting of growth factors, cytokines, enzymes, anti-microbials, and anti inflammatory agents.

13. The matrix according to claim 1 having pores in the size range of 50–300 microns.

14. The matrix of claim 1, wherein the plasma proteins are mixed with the thrombin in the presence of the calcium ions and the at least one anti-fibrinolytic agent under conditions suitable for clotting, optionally with adding of at least one auxiliary component thereto; and the mixture of plasma protiens, thrombin, anti-fibrinolytic agent and optional auxiliary agent are cast upon a solid support prior to clotting; the clotted mixture is frozen; and the clotted mixtire is lyophilized to obtain the matrix.

15. The matrix according to claim 14 wherein the plasma proteins comprise at least fibrinogen and factor XIII.

16. The matrix according to claim 14 wherein at least one of the plasma proteins is autologous.

17. The matrix according to claim 14 wherein all the plasma proteins are autologous.

18. The matrix according to claim 14 wherein the plasma proteins are mixed with at least 0.5 units of thrombin per mg protein.

19. The matrix according to claim 14 wherein the anti-fibrinolytic agent comprises tranexamic acid in an amount of at least 5%.

20. The matrix according to claim 14 wherein the at least one auxiliary component is present and is selected from the group consisting of polysaccharides, anionic polysaccharides, glycosaminoglycans, and synthetic polymers.

21. The matrix according to claim 14 wherein the at least one auxiliary component is present and is selected from the group consisting of hyaluronic acid, pectin, alginate, galactans, galactomannans, glucomannans, polyuronic acids, heparin, chondroitin sulfate, dextran sulfate, dermatan sulfate, heparan sulfate, keratan sulfate, hexuronyl hexosaminoglycan sulfate, inositol hexasulfate, sucrose octasulfate and polyethylene glycol.

22. The matrix according to claim 20 wherein the auxiliary component is present and is dextran sulfate or hyaluronic acid.

23. The matrix of claim 1, wherein the plasma proteins with thrombin in the presence of calcium ions and at least one anti-fibrinolytic agent under conditions suitable for clotting, optionally with adding of at least one auxiliary component; and the mixture of plasma proteins, thrombin, anti-fibrinolytic agent and optional auxiliary agent are cast upon a solid support prior to clotting; the clotted mixture is frozen; and the clotted mixture is lyophilized to obtain a sponge; and the sponge is cut into sections of desired shape; to obtain the matrix; and further the sections are seeded with cells; the cells are grown on the sections until the cells reach a density of at least 104 cells per cm^3; and the seeded sections are implanted in vivo.

24. The matrix according to claim 23 wherein the plasma proteins comprise at least fibrinogen and factor XIII.

25. The matrix according to claim 23 wherein at least one of the plasma proteins is autologous.

26. The matrix according to claim 23 wherein all the plasma proteins are autologous.

27. The matrix according to claim 23 wherein the plasma proteins are mixed with at least 0.5 units of thrombin per mg protein.

28. The matrix according to claim 23 wherein the anti-fibrinolytic agent comprises tranexamic acid in an amount of at least 5%.

29. The matrix according to claim 23 wherein the at least one auxiliary component is present and is selected from the group consisting of polysaccharides, anionic polysaccharides, glycosaminoglycans, and synthetic polymers.

30. The matrix according to claim 23 wherein the auxiliary component is present and is selected from the group consisting of hyaluronic acid, pectin, alginate, galactans, galactomannans, glucomannans, polyuronic acids, heparin, chondroitin sulfate, dextran sulfate, dermatan sulfate, heparan sulfate, keratan sulfate, hexuronyl hexosaminoglycan sulfate, inositol hexasulfate, sucrose octasulfate and polyethylene glycol.

31. The matrix according to claim 23 wherein the auxiliary component is present and is dextran sulfate or hyaluronic acid.

32. The matrix according to claim 23 wherein the at least one auxiliary component is present and is a bioactive agent selected from the group consisting of growth factors, cytokines, enzymes, anti microbials, and anti-inflammatory agents.

33. The matrix according to claim 23 wherein the cells are selected from the group consisting of chondrocytes, hepatocytes, and osteocytes, mesenchymal, epithelial, urothelial, neuronal, pancreatic, renal and ocular cell types.

34. The matrix of claim 1, wherein the plasma proteins are mixed with the thrombin in the presence of the calcium ions and at least one anti-fibrinolytic agent under conditions suitable for clotting, optionally with adding of at least one auxiliary component; and the mixture of plasma proteins, thrombin, anti-fibrinolytic agent and optional auxiliary agent are cast upon a solid support prior to clotting; the clotted mixture is frozen; and the clotted mixture is lyophilized to obtain a sponge having no more than 3% residual moisture; the sponge is optionally washed to remove soluble auxiliary components; optionally the washed sponge is re-lyophilized to reduce the residual moisture to no more than 3%; the sponge is cut into sections of desired shape to obtain the matrix; and the sections of matrix are implanted in situ.

35. The matrix according to claim 34 wherein the plasma proteins comprise at least fibrinogen and factor XIII.

36. The matrix according to claim 34 wherein at least one of the plasma proteins is autologous.

37. The matrix according to claim 34 wherein all the plasma proteins are autologous.

shape

are mixed